3.A.2.3. Antiplaque Efficacy of Stannous Fluoride Dentifrices – Assessed in Digital Plaque Image Analysis Repeated Measures Protocols (Appendix 7)

In the previous sections, the antiplaque effects of stannous fluoride dentifrices were discussed in terms of conventional measures of improved formulations in long term plaque and gingivitis clinical studies and in an accelerated experimental gingivitis model. During the period since the Plaque Subcommittee deliberations and publication of the ANPR, additional studies have been carried out supporting the antiplaque effects of original and improved stannous fluoride dentifrices in P&G laboratories. These studies take advantage of a technique originating from state of the art image analysis capabilities. In repeated studies with this highly sensitive and specific clinical technique, stannous fluoride dentifrices have demonstrated significant and reproducible efficacy in reducing plaque accumulation *in vivo*.

Research has shown that Digital Plaque Image Analysis Repeated Measures (DPIARM) presents a unique and sensitive objective (instrumental) methodology to evaluate the ability of mouthwashes and dentifrices to remove, prevent or control plaque accumulation on the surfaces of teeth *in vivo*. Its utility is a quantitative assessment of plaque that is devoid of subjective grading and is not influenced by confounders such as extrinsic tooth staining, gingival appearance or thickened pellicle formation.

Sodor et. al. in 1993 published preliminary evidence applying a planimetric imaging method to plaque assessments along the gumline. The rationale was simple. The majority of plaque development of clinical consequence is isolated along the gingival margin and in interproximal gingival regions (near the papilla). These plaque biofilms, though most important to disease progression, are difficult to enumerate in categorical indices – such as Turesky or Löe Silness, where variable levels of plaque at the gingival margin are scored similarly in average patients with typical oral

hygiene (the target population for antigingivitis and antiplaque products). In the exploratory phase of imaging studies, researchers chose various disclosure media for the plaque and used photography and hand planimetric analysis[52] and later digital imaging[53] to enumerate plaque coverage on teeth and the longitudinal consistency of plaque formation as determined with the method. The quantitative assessment of the plaque was typically developed through a combination of computer image analysis selection followed by area determinations, though the selection criteria for differentiation were usually empirically or subjectively derived. These techniques clearly demonstrated improved sensitivity of plaque quantitation at the most ideal site of analysis, the gingival margin, though the subjective nature of selection of plaque covered areas and the laborious requirement for individual image analysis limited applications[54]. Sagel et al., in 1995, reported on the application of a custom digital imaging system within the Procter & Gamble laboratories to plaque disclosure evaluations *in vivo* using an objective and quantitative decision rule (rather than a subjective scale) for the selection of plaque coverage. An algorithm was developed to allow automated and objective digital imaging quantitative selection of plaque free and plaque covered tooth surfaces chosen by color representation in RGB (red, green, blue) - color space. In internal studies, White and Sagel selected a clinical protocol where proportional efficacy would be compared as the proportion of plaque coverage of teeth – analogous, albeit in an objective quantitative technique with improved sensitivity and specificity, to the categorical and subjective indices used for conventional plaque assessments. White and coworkers further refined the

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[52] Soder PO, Lin LJ, Soder B: Computerized planimetric method for clinical plaque measurement. *Scand J Dent Res* 101: 21-25, 1995.

[53] Soder B, Lin LJ, Lundquist G, Soder PO: A longitudinal investigation of the indivudal consistency of plaque levels in adults. *Acta Odont Scand* 53: 72-74, 1995.

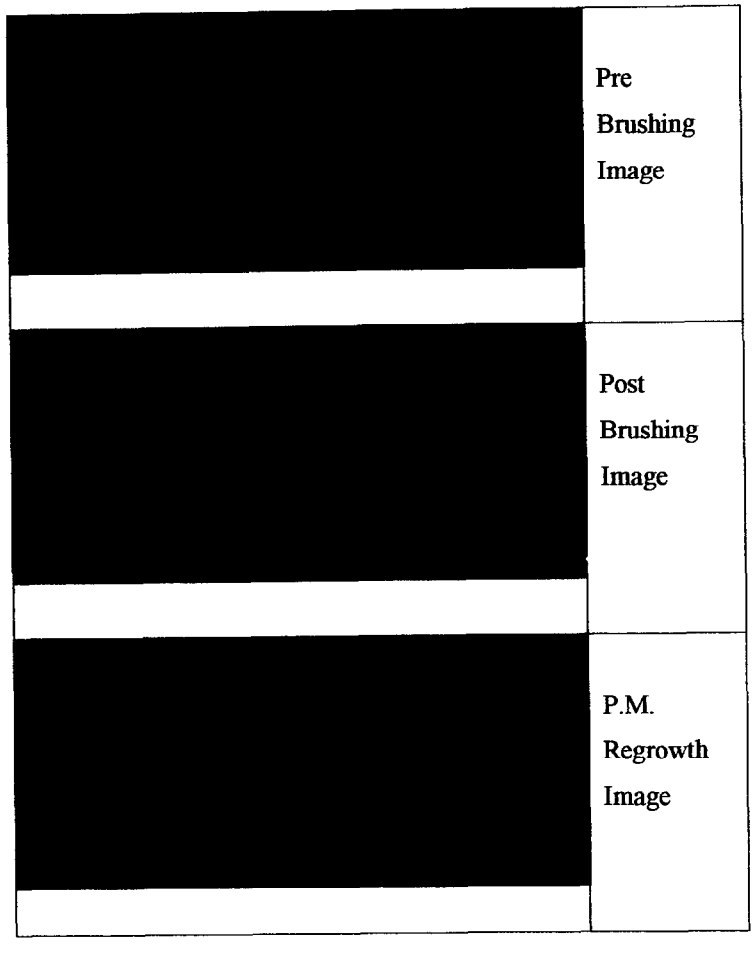
[54] Sagel PA, Lapujade PG, Miller JM, Sunberg RJ: Objective quantification of plaque using digital image analysis. Monographs in Oral Science, Assessments of Oral Health, RV Faller (ed), Basel, Karger, pp130-143, 2000.

methodology including the controlled assessment of plaque development at specific time points during the day, at defined intervals vs. oral hygiene. Lastly, White and coworkers included the adoption of repeated measures of plaque for clinical assessments in order to compensate for significant day-to-day variations in plaque accumulation rates for subjects. The refined 'Digital Plaque Image Analysis Repeated Measures" (DPIARM) method has proved to be a valuable addition to research methods directed toward the assessment of antimicrobial benefits in the P&G laboratories.

As described, the DPIARM has a beneficial feature in that the excellent sensitivity and specificity for plaque determinations, permits assessments of plaque at various times during the day, providing perspective on both antibacterial efficacy and comparative benefits to plaque insult vs. conventional hygiene measures. Figure 2 shows representative DPIA screens of plaque as measured in the morning (before brushing), immediately following morning hygiene and in the late afternoon – some 6 hours since last toothbrushing.

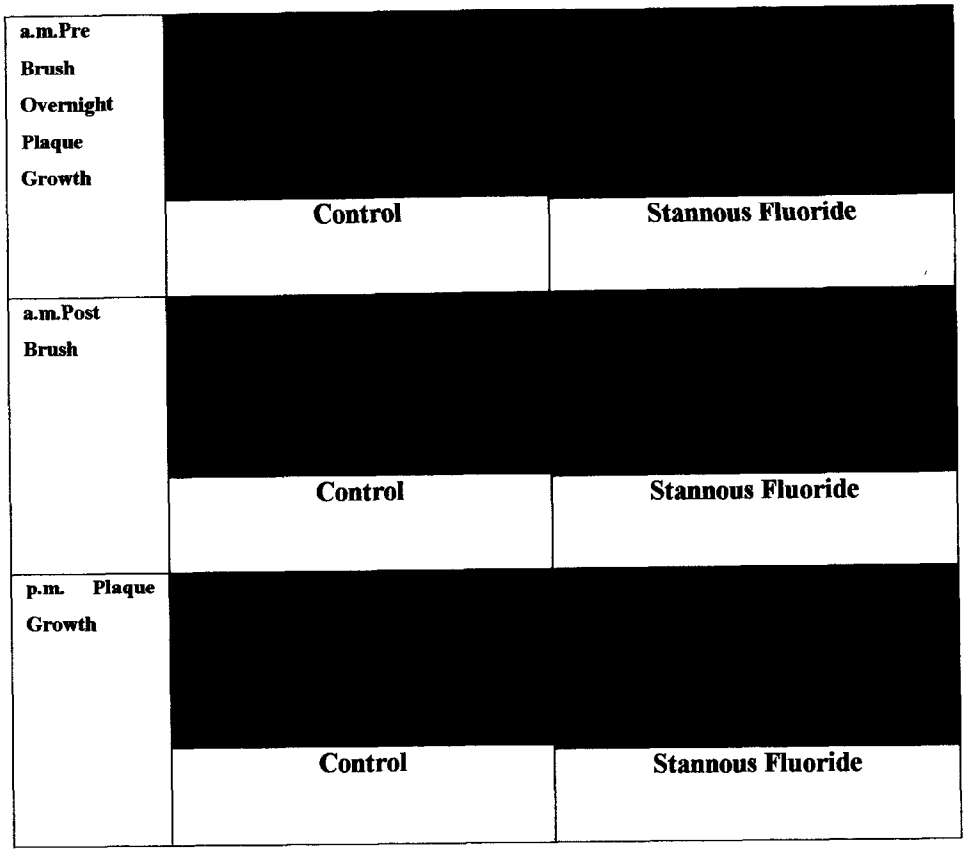
Plaque Development Patterns on Facial Surfaces at Diurnal Measuring Points as Assessed by Digital Imaging

Figure 2



As shown, the DPIA easily distinguishes diurnal plaque development and effects of hygiene. Figure 3 illustrates typical antimicrobial effects observed at these same measuring times during the day.

Figure 3



The primary effect of antimicrobials is suppression of plaque formation in the intervals between hygiene interventions – here shown as differences in plaque formation at various diurnal measuring points.

We have applied DPIA in our laboratories to the screening of multiple stannous fluoride dentifrices. Appendix 6 shows a typical protocol for DPIARM testing. Unique to the DPIA protocol is the ability to assess plaque regrowth at selected time points, with a morning toothbrushing intervention included. The table below reveals results obtained at primary antibacterial screening endpoints – a.m. pre brushing and p.m. plaque regrowth respectively.

Antiplaque Effects of Stannous Fluoride Dentifrices in Six DPIA Studies – Numerical Magnitude of Plaque Benefits in Studies over 2 Years

% Reduction vs Crest Cavity Protection – a.m. Plaque Regrowth	% Reduction vs Crest Cavity Protection – p.m. Plaque Regrowth
24.4	27.9
11.6	16.0
30.3	34.9
24.4	22.5
12.8	11.5
23.4	24.5

The table clearly highlights substantial and reproducible efficacy of stannous fluoride dentifrice in producing reductions in plaque accumulation *in vivo*. For reference purposes in the table, DPIA plaque inhibition benefits of 15% can be characteristically determined as significantly different from controls using standard ANCOVA for repeated measures. As shown, stannous fluoride dentifrices have been shown to exhibit strong antimicrobial efficacy in these plaque panels – ranging anywhere from 12-35 % depending upon the individual study. These results are similar to plaque protection provided by other marketed antimicrobial formulations (triclosan, essential oils) and provide further basis to support a general antiplaque effect for stannous fluoride dentifrices.

Overall, the results of DPIARM testing of stannous fluoride dentifrices show greater activity than observed in longer term clinical trials. The differences in sensitivity to stannous fluoride effects may be due to several factors, including:

1. **Superior specificity of imaging as a plaque assessment tool:** The DPIA uses imaging as a plaque assessment methodology, with a plaque specific dye (fluorescein) and rigorous analytical differentiation of plaque vs. other intraoral deposits – Net: DPIA imaging may be superior to visual/tactile grades – particularly in the presence of stains or other artifacts and particularly in patients where the majority of plaque is present along the gingival margin;

2. **Superior sensitivity of DPIA due to control of plaque analysis time:** The DPIA uses a non-invasive or non-interfering technique to assess plaque levels *in vivo*. The technique is rapid and non invasive, hence permits the controlled assessment of plaque formation at defined diurnal intervals. In contrast, conventional plaque evaluations in a clinical population attempts to discern antimicrobial benefits in a background of uncontrolled hygiene and regrowth periods, no doubt decreasing sensitivity.

3. **DPIA may be more sensitive to 'real' plaque levels:** The specificity and sensitivity of the imaging evaluations permit assessments of antiplaque effects in normal populations, including typical patients with only modest plaque accumulations. In conventional clinical trials, the gingivitis incidence entrance criteria tend to select for low hygiene patients, in which differentiation of plaque regrowth inhibition may be difficult.

In any case, DPIARM would appear to be an improved methodology for the quantitative enumeration of antiplaque efficacy of oral topical ingredients. The model has shown sensitivity for differentiating the effects of conventional and aggressive oral hygiene, as well as antibacterial effects on plaque regrowth. Results of PGRM testing confirm that stannous fluoride dentifrice is effective for prevention of plaque accumulation *in vivo* and warrants an indication to this effect in product labeling.